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2. Patent application number *(The Patent Office will fill in this pa* — 0327906.4

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Ciba Specialty Chemicals Water Treatments Limited
Cleckheaton Road
Low Moor
Bradford
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BD12 OJZ

Patent ADP number *(if you know it)* — 7585391005

If the applicant is a corporate body, give the country/state of its incorporation — England

4. Title of invention — BIOCATALYST COMPOSITION

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Patents ADP number *(if you know it)* — 07585391002

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a) any applicant named in part 3 is not an inventor, or

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11. I/We request the grant of a patent on the basis of this application

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Date: 02 December 2003

12. Name and daytime telephone number of person to contact in the United Kingdom: Jane Spinks 01274 417558

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Biocatalyst Composition

The present invention relates to biocatalyst compositions especially containing nitrile hydratase enzyme and in particular to a method of storage of biocatalyst at ambient temperatures, especially nitrile hydratase biocatalysts. The invention also relates to a novel nitrile hydratase enzyme and also to a method of converting a nitrile to an amide employing a biocatalyst that contains a nitrile hydratase.

It is well known to employ biocatalysts, such as microorganisms that contain enzymes, for conducting chemical reactions. Nitrile hydratase enzymes are known to catalyse the hydration of nitriles directly to the corresponding amides. Typically nitrile hydratase enzymes can be produced by a variety of microorganisms, for instance microorganisms of the genus Bacillus, Bacteridium, Micrococuss, Brevibacterium, Corynebacterium, Pseudomonas, Acinetobacter, Xanthobacter, Streptomyces, Rhizobium, Klebsiella, Enterobacter, Erwinia, Aeromonas, Citrobacter, Achromobacter, Agrobacterium, Pseudonocardiaand Rhodococcus.

Various strains of the Rhodococcus rhodochrous species have been found to very effectively produce nitrile hydratase enzyme. . EP-0 307 926 describes the culturing of Rhodococcus rhodochrous, specifically strain J1 in a culture medium that contains cobalt ions. The nitrile hydratase can be used to hydrate nitriles into amides, and in particular the conversion of 3-cyanopyridine to nicotinamide. Rhodococcus rhodochrous J1, is used commercially to manufacture acrylamide monomer from acrylonitrile and this process has been described by Nagasawa and Yamada Pure Appl. Chem. 67: 1241-1256 (1995). EP-A-0362829 describes a method for cultivating bacteria of the species Rhodococcus rhodochrous comprising at least one of urea and cobalt ion for preparing the cells of Rhodococcus rhodochrous having nitrile hydratase activity. Specifically described is Rhodococcus rhodochrous J1.

Leonova et al., Appl. Biochem. Biotechnol. 88: 231-241 (2000) entitled, "Nitrile Hydratase of Rhodococcus", describes the growth and synthesis of nitrile hydratase in Rhodococcus rhodochrous M8. The NH synthesis of this strain is induced by urea in the medium, which is also used as a nitrogen source for growth by this organism. Cobalt is also required for high nitrile hydratase activity. This literature paper looks at induction and metabolic effects in the main.

Leonova et al., Appl. Biochem. Biotechnol. 88: 231-241 (2000) also states that acrylamide is produced commercially in Russia using Rhodococcus rhodochrous M8. Russian patent 1731814 describes Rhodococcus rhodochrous strain M8.

Rhodococcus rhodochrous strain M33 that produces nitrile hydratase without the need of an inducer such as urea is described in US-A-5827699. This strain of microorganism is a derivative of Rhodococcus rhodochrous M8.

In order to achieve maximum productivity from the biocatalyst it is important that the maximum biocatalytic activity is retained during its preparation and storage prior to use. In Chaplin and Bucke (1990) In: Enzyme Technology, published by Cambridge University Press, p 47 (Enzyme preparation and use) it was recognised that enzyme inactivation can be caused by heat, proteolysis, sub optimal pH, oxidation denaturants and irreversible inhibitors. A number of substances may cause a reduction in the rate of an enzymes ability to catalyse a reaction. This includes substances that are non-specific protein denaturants, such as urea.

In the presentation, Protein Stability, by Willem JH van Berkel, Wageningen University, factors that may cause deactivation or unfolding were considered and these included proteases, oxidation due to the presence of oxygen or

oxygen radicals and denaturing agents causing reversible unfolding, such as urea.

Chaplin and Bucke (1990) in Enzyme Technology, published by Cambridge University Press, p 73 (Enzyme preparation and use) revealed that the key factor regarding the preservation of enzyme activity involves maintaining the conformation of the enzyme structure. Therefore it was considering important to prevent unfolding, aggregation and changes in the covalent structure. Three approaches for achieving this were considered: (1) use of additives; (2) the controlled use of covalent modification; and (3) enzyme immobilisation.

WO-A-02/50297 describes using a microbial catalyst for converting a nitrile into an amide in which cells of a microorganism having relatively high nitrile hydratase activity are contacted with a nitrile. The cells of the microorganism have a nitrile hydratase activity of 50 U or more per mg of dry cells and the reaction is carried out at a temperature of 10°C without entrap-immobilisation of the microorganism cells. No mention of biocatalyst storage is made however.

US-A-4,931,391 describes a method for the preservation of nitrile hydration activity by adding as a stabiliser compounds selected from nitriles, amides and organic acids and their salts to a solution or suspension of the enzyme. It was described in EP-A-0 707 061 that addition of inorganic salts at a concentration of between 00 mM to the saturation concentration of the inorganic salts to an aqueous medium containing either a suspension of microbial cells or immobilized microbial cells preserved the cells and enzyme activity for a prolonged period of time. The addition of bicarbonate or carbonate salts to an aqueous solution of immobilized or unimmobilised microbial cells having nitrilase activity is described in US-B-6,368,804.Immobilisation has frequently involved removal of the enzyme from the whole cell, before immobilising the enzyme in a matrix. However, although such immobilisation provides very good

protection for the enzyme, extraction of the enzyme from the whole cell is an intricate step, which can be time-consuming, expensive and can lead to loss of enzyme. Additionally whole microbial cells can be immobilized. US-A-5,567,608 provides a process of immobilising whole cell biocatalyst in a cationic copolymer which has good storage stability and prevents putrefication.

Rhodococcus rhodochrous J1, which is used commercially to manufacture acrylamide monomer, is immobilised to (a) allow transportation and (b) to increase the longevity of the biocatalyst in use. In US-A-5,567,608 the inventors state that biocatalysts are normally immobilized for use on an industrial scale, to facilitate ease of separation of the biocatalyst from the reaction product, preventing impurities from the biocatalyst eluting into the product and to assist in continuous processes and recycling of the biocatalyst. However, immobilisation is an extra processing step that requires an additional plant and the use of potentially a number of other raw materials such as alginate, carrageenan, acrylamide and other acrylate monomers, and vinyl alcohol. Thus, this is an expensive processing step.

Various other ways have been proposed for minimising the deleterious effects of enzyme inactivation in an attempt reduce the negative impact on a chemical reaction process.

In US-A-6,043,061 it was revealed that the reduction of hydrocyanic acid concentration in the reaction mixture can suppress the deactivation of nitrile hydratase.

It is also known to freeze dry biocatalysts in order to preserve the activity of an enzyme in storage over prolonged period of time. Again this is a potentially expensive processing step that is normally carried out with biocatalysts prepared on a small scale. Cryopreservation in liquid nitrogen or in the vapour

phase of liquid nitrogen also affords long-term storage of microbial cells but requires a constant supply of liquid nitrogen.

Growth of a microorganism for use as a biocatalyst may take place over a period of several days. During this time the microorganism is actively growing and is maintained in a state of growth by the feeding of appropriate nutrients and maintaining a correct temperature and pH for growth and supplying oxygen if required.

There are instances where the biocatalyst has ceased its growth period but it is required to continue metabolizing for it to be an active biocatalyst. For instance for co-factor regeneration for a biocatalysed reaction to occur and in these cases compounds are fed to the biocatalyst to maintain the metabolism.

However, if a biocatalyst, such as one that produces nitrile hydratase is to be stored without continued growth for a period of time, even for a few days it is normal to remove the microbial cells from the fermentation broth, whether it is the cells that are required as the catalyst, or whether the enzyme is secreted into the fermentation medium. This is to prevent microbial growth in the fermentation broth causing putrefaction of the broth and to reduce protease activity that can cause the breakdown of the enzyme that is required. It is normal therefore to preserve the fermentation broth *per se* or to remove the cells to prevent the degradation of the biocatalyst through extraneous biological activity such as microbial contamination. The biocatalytic activity could normally be expected to reduce in a very short period of time such as within a day and certainly in less than two days if this were not carried out. Methods of preserving the activity during the storage of biocatalysts, even for periods of time up to one-week, have normally involved removal of the biocatalyst from the fermentation broth and/or immobilisation of the biocatalyst in a suitable matrix and/or stabilisation using stabilising substances which then either become contaminants in the reaction mixture and this may be a problem or that they

require an additional processing step to remove them from the microbial cell suspension before it is used as a biocatalyst.

In the absence of such treatments biocatalysts when kept at ambient temperatures tend to lose activity to the extent that they are no longer as effective or even suitable for catalysing reactions.

It would be desirable to provide a method of storing biocatalysts especially comprising nitrile hydratase enzyme without any significant loss of activity over a period of above 2 days, for instance 5 days, a week or more which avoids the additional processing steps normally required to achieve storage stability. It would be desirable to provide biocatalyst, especially a nitrile hydratase biocatalyst composition that does not putrefy on storage at ambient temperatures. It would also be desirable to provide a biocatalyst composition, in particular a nitrile hydratase biocatalyst composition that is capable of being stored without losing activity. Furthermore, it would be advantageous to provide a composition or storage method in which the activity of the biocatalyst actually improved. It would be particularly desirable to provide an improved method of catalysing the reaction in which a nitrile is hydrated to the corresponding amide.

According to the present invention we provide a composition comprising a biocatalyst that is in the form of a non-actively growing free cell microorganism in a fermentation broth,
wherein the composition comprises urea or a derivative of urea.

We also provide a method of storing a biocatalyst that is in the form of a non-actively growing free cell microorganism in a storage medium that comprises a fermentation broth,
wherein the storage medium comprises urea or a derivative of urea.

The microbial cells may be regarded as a non-actively growing culture. By this we mean that the medium and the storage conditions in which the microorganism is held would not be expected to promote growth. The storage medium can for instance be a growth medium where metabolism in the microorganism cells is substantially zero as determined by measuring the growth rate, or the biomass concentration or oxygen consumption or nutrient consumption, or other form of measurement generally used to monitor microbial growth and metabolism.

Growth of a microorganism for use as a biocatalyst may take place over a period of several days. During this time the microorganism is actively growing, that is to say balanced growth where the biomass is increasing together with an increase in and maintenance of the overall chemical composition of the cell.

Normally the growth of microorganisms is limited either by the exhaustion of nutrient or the accumulation of toxic products of metabolism and the growth rate reduces. Growth is maintained by feeding appropriate nutrients and maintaining a correct temperature and pH for growth and where required supplying oxygen.

The storage method promotes effective stability such that the biocatalyst can be readily used without any significant loss in activity. Storage stability is achieved without the necessity of resorting to for instance immobilisation, freeze drying or removal of the urea, even though urea is a known protein deactivator.

The biocatalyst may for instance be a microorganism that is capable of producing an amidase or a nitrilase, but preferably it is a microorganism capable of producing a nitrile hydratase.

The composition or the environment used in the method of storage may contain oxygen or can be a substantially oxygen free environment. By oxygen free we mean that the concentration of oxygen should be less than 1% dissolved

oxygen concentration Removal of oxygen from the fermentation broth can be achieved by any of the conventional methods for removing oxygen. These include purging for a period of time with an inert gas, removal of any head-space in the storage container, storing under diminished pressure or the addition of known oxygen scavengers such as ascorbic acid or hydrazine and hydrazide.

It would have been expected that after 2 days and especially after several days storage, even in the absence of oxygen, but especially in the presence of urea at above 0°C there would be some loss in nitrile hydratase activity. This is because protease enzymes in the biocatalyst might be expected to break down other proteins in the cell, including the nitrile hydratase. Furthermore, the presence of urea or urea derivative could be expected to be detrimental, since urea is known to be a protein deactivator. However, the biocatalyst suffers none of the expected disadvantages and thus suffers no significant loss in nitrile hydratase activity. On the contrary we find that during the storage period the activity of the biocatalyst comprising nitrile hydratase actually increases. Thus in another aspect of the invention we provide a method of increasing the nitrile hydratase activity of a biocatalyst capable of forming nitrile hydratase by storing the biocatalyst in a storage medium in accordance with the storage method of the present invention. Therefore, the method can result in a new biocatalyst composition by virtue of its increased activity. Therefore, nitrile hydratase of the biocatalyst composition, and in particular formed during storage of the biocatalyst is new. Also, the biocatalyst does not produce the mal odours associated with putrefaction during the storage period.

Preferably the storage method allows the biocatalyst to be stored for at least two days and more preferably one or more weeks. In particular the biocatalyst may be stored from three to fourteen days.

The urea derivative can be for example an alkyl derivative of urea. Preferably though it is urea which is employed. Typically urea or the urea derivative may be included in the biocatalyst composition of biocatalyst storage environment in an amount of at least 0.1 g/L. The amounts can be as much as 15 g/L or even higher but usually it will be up to 9 or 10 g/L. Preferably the concentration of the urea will be in the range 2 to 8 g/L. More preferably this will be3 to 6 g/l.

Urea or the urea derivative is present in the biocatalyst composition through its inclusion in the fermentation mixture. In one form of the invention of the preferably deoxygenated fermentation mixture containing the nitrile hydratase biocatalyst and urea is the composition or storage environment in accordance with the present invention.

The biocatalyst is desirably a microorganism which is capable of generating nitrile hydratase enzyme. For instance this could be a microorganism selected from the genus Bacillus, Bacteridium, Micrococuss, Brevibacterium, Corynebacterium, Pseudomonas, Acinetobacter, Xanthobacter, Streptomyces, Rhizobium, Klebsiella, Enterobacter, Erwinia, Aeromonas, Citrobacter, Achromobacter, Agrobacterium, Pseudonocardia and Rhodococcus. The biocatalyst is especially a microorganism of the Rhodococcus genus, preferably of the Rhodococcus rhodochrous species. A particularly suitable biocatalyst is Rhodococcus rhodochrous rhodochrous strain 2368 (NCIMB 41164) which is described and claimed in our co-filed UK patent application which has been allocated case reference number BT/3-22351/P1.

Rhodococcus rhodochrous strain 2368

1. Origin and Deposition

The strain 2368 was isolated by us from soil in Bradford, England and deposited on 5th March 2003 at the National Collection of Industrial and Marine Bacteria (NCIMB), where it was assigned the accession number NCIMB 41164 under the Budapest Treaty.

2. Morphological and cultural characteristics
   (1) Polymorphic growth
   (2) Motility: immotile
   (3) Non-spore former
   (4) Gram positive
   (5) Aerobic
   (6) Growth on nutrient agar gives salmon pink round colonies within 48 hours at 30°C

A particularly advantageous feature of the present invention is that it is no longer necessary to separate the biocatalyst from the fermentation mixture in which it was cultured. This is of significant value since it avoids the requirement for an additional processing step. Therefore the composition may also comprise a fermentation mixture, which is then stored. In the method of storing the biocatalyst, we find that this may also be achieved in the presence of a fermentation mixture without any detrimental effects on the activity of the enzyme. This then allows the fermentation broth to be used immediately to catalyse the reaction, or to allow it to be stored for several days or even weeks without detriment whilst the bioconversion step is being carried out also over a period of several days, thus ensuring a constant supply of readily available biocatalyst without need for additional processing steps thus simplifying and reducing the cost of the bioconversion step.

The biocatalyst may conveniently be stored at temperatures above 0°C. Typically the biocatalyst may be stored at ambient temperatures, for instance up to 30 or 40°C. However, the advantage of the present method is that the biocatalyst may be stored at ambient temperatures without any special precautions for monitoring and controlling the temperature. Preferably the biocatalyst is stored at a temperature between 4 and 30°C, more preferably between 5 and 25°C, in particular 10 to 15°C.

According to a further aspect of the present invention we provide a method of producing an amide by contacting the corresponding nitrile by a nitrile hydratase,
in which the biocatalyst is part of a composition or stored in the form of a non-actively growing free cell microorganism in a storage medium in which the composition or storage medium comprises fermentation broth,
wherein the composition or storage medium comprises urea or a derivative of urea.

Thus in accordance with this aspect of the invention the biocatalyst is for instance a microorganism capable of producing a nitrile hydratase enzyme and it may have been held in an environment containing oxygen or held in an oxygen-free environment containing a urea prior to commencing the conversion of the nitrile. This may be resulting from storing the biocatalyst in accordance with the present invention or alternatively provided as a composition in accordance with the present invention.
The type of biocatalyst is as described previously in connection with the present invention.

As given previously it is not necessary to remove the biocatalyst from the fermentation mixture in which the biocatalyst has been prepared. Thus in a preferred form the environment in which the biocatalyst is held also contains components of a fermentation broth. Therefore a biocatalyst composition containing urea and components of a fermentation broth can be combined with a nitrile which is then hydrated to the corresponding amide. We have found surprisingly that in contrast to previous knowledge for instance in US-A-5,567,608 states that immobilisation of the biocatalyst is preferable to prevent elution of impurities from the biocatalyst into the reaction product, that the inclusion of fermentation broth in the reaction mixture does not affect the quality of the final product and this aspect is described in our co-filed application, identified by case number BT/3-22349/P1 .

The fermentation mixture will comprise essential components for allowing microorganisms to be grown and sustained. In general the mixture will at least contain a carbon source, nitrogen source and various nutrients. This may include a monosaccharide such as glucose or other sugar, ammonium salts, complex medium components such as yeast extract and peptone, amino acids, vitamins, phosphate salts, potassium, sodium, magnesium and calcium salts, trace elements such as iron, cobalt, manganese,copper, zinc and the like. These and other ingredients can be included in the fermentation mixture at concentrations suitable for the particular microorganism. It is known that fermentations can be subject to changes in the productivity of the biocatalyst and the fermentation broth may be used at different stages of growth and so it is important to be able to store the biocatalyst after production in such a way.

We find that the activity of the biocatalyst does not diminish significantly during the reaction for a prolonged period. Consequently the biocatalyst may be replaced less frequently. Preferably the biocatalyst is used for a period of at least 2 days and loses substantially no activity over that period.

Generally the catalysis of the reaction using nitrile hydratase enables the nitrile to be converted into the corresponding amide in a single step. This process is a particular value when the nitrile is acrylonitrile and the amide is acrylamide. It is desirable to carry out this conversion step several times using one batch of biocatalyst. Thus, it is important to be able to store the biocatalyst as inexpensively as possible without detriment to the catalyst whilst the bioconversion step is carried out simultaneously. So in effect one batch of biocatalyst can be used to make several batches of for instance acrylamide. Several batches could be from 5 to 10 or more batches, even 15 to 20 batches.

The following examples are an illustration of the invention.

Example 1

(1)Rhodococcus rhodochrous NCIMB 41164 was grown in a 280L fermenter containing 180 L culture medium containing the following constituents (g/L): diPotassium hydrogen phosphate 0.7; Potassium hydrogen phosphate 0.3; glucose 1.0; yeast extract 3.0; magnesium sulphate heptahydrate 0.5; cobalt chloride hexahydrate 0.01; urea, 5.0. The pH of the medium was adjusted to pH 7.2. The culture was grown at 30°C for 3 days.

25L of the fermentation broth was degassed with nitrogen for 20 minutes prior to storage at ambient temperature, which was approx. 5°C for 3½ days. The nitrile hydratase activity was measured 15 h after harvesting and it was found to be 242,000 U/g at 25°C. When the NH activity was re-measured immediately prior to the first acrylamide production trial 3 days later it was found to be 293,000 U/g.

Example 2

Rhodococcus rhodochrous NCIMB 41164 was grown in a 2 L Erlenmeyer flask for 5 days at 28°C with shaking at 180 rpm in a culture medium containing the following constituents in g/L: diPotassium hydrogen phosphate 0.7; Potassium hydrogen phosphate 0.3; glucose 10.0; yeast extract 3.0; urea 5.0; magnesium sulphate heptahydrate 0.5; cobalt chloride hexahydrate 0.01;. The pH of the medium was adjusted to pH 7.2. The bacterial cells from half of the culture broth was harvested using centrifugation. The culture broth was divided into two portions, one half of which was deoxygenated using nitrogen for 10 minutes. Portions of both the deoxygenated and the oxygenated culture broth were incubated at 4, 15 and 25°C for 1 week. The nitrile hydratase activity of the portions was measured periodically.

The results of the nitrile hydratase assays are shown in Table 1. The results are given in U/mg dry cells

Table 1

Incubation temp.	Time (days)					
	0	1	2	3	5	7
4°C (O2)	140	286		232	267	257
4°C (degassed)		274			214	293
15°C (O2)						
15°C (degassed)	140	218				
25°C (O2)	140	143				
25°C (degassed)		154	230			

Example 3

Rhodococcus rhodochrous J1 was grown in a 2 L Erlenmeyer flask for 5 days at 28°C with shaking at 180 rpm in a culture medium containing the following constituents in g/L: diPotassium hydrogen phosphate 0.5; Potassium hydrogen phosphate 0.5; glucose 20.0; peptone, 5.0; yeast extract 1.0; urea 7.5; magnesium sulphate heptahydrate 0.5; cobalt chloride hexahydrate 0.01;. The pH of the medium was adjusted to pH 7.2. The bacterial cells from half of the culture broth was harvested using centrifugation. The culture broth was divided into two portions, one half of which was deoxygenated using nitrogen for 10 minutes. Portions of both the deoxygenated and the oxygenated culture broth were incubated at 4, 15 and 25°C for 1 week. The nitrile hydratase activity of the portions was measured periodically.

Incubation temp.	Time (days)					
	0	1	2	3	5	7
4°C (O2)	78	86			87	78
4°C (degassed)		92		101	90	73
15°C (O2)						
15°C (degassed)	78	94				
25°C (O2)	78	96				
25°C (degassed)		90	86			

It can be seen from the results of both Examples 2 and 3 and that the biocatalyst can be stored effectively at ambient temperatures. Furthermore it can be seen that the nitrile hydratase activity does increase on storage by comparison to day zero. This was most noticeable for Rhodococcus rhodochrous NCIMB 41164.

Claims

1. A composition comprising a biocatalyst that is in the form of a non-actively growing free cell microorganism in a fermentation broth, wherein the composition comprises urea or a derivative of urea.

2. A composition according to claim 1 in which the biocatalyst is a microorganism that is capable of producing nitrile hydratase.

3. A composition according to claim 1 or claim 2 in which the biocatalyst is a microorganism of the Rhodococcus genus, preferably of the Rhodococcus rhodochrous species.

4. A composition according to claim 3 in which the biocatalyst is Rhodococcus rhodochrous NCIMB 41164.

5. A method of storing a biocatalyst that is in the form of a non-actively growing free cell microorganism in a storage medium that comprises fermentation broth, wherein the storage medium comprises urea or a derivative of urea.

6. A method according to claim 5 in which the biocatalyst is stored at a temperature above 0°C, preferably between 4 and 30°C.7. A method according to claim 5 or claim 6 in which the biocatalyst is stored for a period of at least two days, preferably between 3 and 14 days.

8. A method according to any of claims 5 to 7 in which the biocatalyst is a microorganism that is capable of producing nitrile hydratase.

9. A method according to any of claims 5 to 8in which the biocatalyst is a microorganism of the Rhodococcus genus, preferably of the Rhodococcus rhodochrous species.

10. A method according to claim 9 in which the biocatalyst is Rhodococcus rhodochrous NCIMB 41164.

11. A composition obtainable by the method according to any of claims 5 to 10.

12. A nitrile hydratase obtainable from the composition according to any of claims 1 to 4 or obtainable by the method according to any of claims 5 to 10.

13. A method of increasing the nitrile hydratase activity of a biocatalyst that is capable of producing nitrile hydratase comprising
storing the biocatalyst that is in the form of a non-actively growing free cell microorganism in a storage medium that comprises fermentation broth, wherein the storage medium comprises urea or a derivative of urea.

14. A method according to claim 13 in which the biocatalyst is a microorganism of the Rhodococcus genus, preferably of the Rhodococcus rhodochrous species.

15. A method according to claim 14 in which the biocatalyst is Rhodococcus rhodochrous NCIMB 41164.

16. A method according to any of claims 13 to 15 in which the biocatalyst is stored for a period of at least two days, preferably between 3 and 14 days.

17. A method of producing an amide by contacting the corresponding nitrile by a nitrile hydratase, wherein the nitrile hydratase is obtainable from a composition according to claims 1 to 4 or obtainable by a method according to claims 5 to 11.

18. A method according to claim 17 in which the amide is (meth)acrylamide.

Abstract

Biocatalyst Composition

A composition comprising a biocatalyst that is in the form of a non-actively growing free cell microorganism in a fermentation broth, wherein the composition comprises urea or a derivative of urea. A method of storing a biocatalyst that is in the form of a non-actively growing free cell microorganism in a storage medium that comprises fermentation broth, wherein the storage medium comprises urea or a derivative of urea. The biocatalyst composition exhibits no significant loss of activity, for example if stored for at least two days, especially 3 to 14 days. A method of increasing the nitrile hydratase activity of a biocatalyst. A method of producing an amide from the corresponding nitrile and using a nitrile hydratase and obtainable from the biocatalyst composition or from the method of storing a biocatalyst.

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